Necator americanus in the DSN hamster: density-dependent expulsion of adult worms during primary infection

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SUMMARY

Neonatal hamsters were exposed to varying doses of *Necator americanus* larvae and changes in the stability of the resulting worm burdens were monitored over a period of 25 weeks. No change in worm burdens was evident for the first 5 weeks of infection, irrespective of the infection intensity, but the more heavily infected groups subsequently lost worms in a density-dependent manner. Male and female hamsters lost comparable proportions of their established parasite burdens indicating that there was no host sex-linked difference in this respect. By week 15 infections had stabilized and the residual worm burdens, usually a maximum of 30 worms survived for a considerably longer period of time. Initially the percentage of male worms varied from 45% to 50% but as infection progressed male worms comprised a significantly increasing proportion of the total worm burden. By week 25 the percentage of male worms was usually in excess of 60%. The growth of infected animals was not severely affected by *N. americanus*, even when heavy worm burdens established initially, but a significant effect was detected particularly in week 5, prior to worm loss, when the adult worms would have been feeding on intestinal tissues and causing blood loss for a period of about 2 weeks. The most severe depression in the packed cell volume was also recorded in week 5, indicating that anaemia had been initiated in infected hamsters. Whilst, the regulation of parasite burdens in weeks 5–10 post-infection may have resulted from host immunity, the persistence of the residual worm burdens, the marked density-dependent anaemia and the subtle effect on host weight, all reflected well-documented aspects of chronic human necatoriasis.

Key words: *Necator americanus*, hookworms, nematode, hamster, worm loss, anaemia, weight loss, density-dependent expulsion.

INTRODUCTION

Necator americanus, one of the three species of hookworms which mature in humans, is still wide spread on a global scale and is an important pathogen of man in tropical countries (Rep, 1964; Banwell & Schad, 1978; Crompton, 1989). The pronounced host specificity of this parasite has hindered progress in the understanding of its biology, and hence little is known about the nature of potential host-protective immune responses and about the strategies employed by the worms to circumvent immunity (Behnke, 1987). However, *N. americanus* has been successfully adapted to maintenance in laboratory hamsters (Sen & Seth, 1967) but the model has remained poorly characterized. The development of adult worms in hamsters is dependent on exposure of neonatal animals to infective larvae because only a few worms establish in the intestine when adult animals are infected (Rajasekariah *et al.* 1985). Sen (1972) showed that the worms developing in neonatal hamsters become fecund and that egg production was sustained for over 100 days post-infection in male hamsters. Subsequently Behnke & Pritchard (1987) reported that substantial numbers of adult *N. americanus* were lost by day 100, that the proportion of worms lost was dependent on the number initially established and that some worms survived for 270 days after infection. In this paper we present the results of experiments undertaken to relate the dose-dependent nature of parasite loss to changes in packed cell volume (PCV) and growth following infection during the neonatal period.

MATERIALS AND METHODS

Animals

Inbred DSN hamsters (*Mesocricetus auratus*) were purchased from Intersimian Ltd, Oxford, UK, to set up a breeding colony, maintained under conventional animal house conditions. Groups of female hamsters were mated 18–20 days before the day of infection to provide litters 1–3 days old, which were subsequently weaned from the mother at 3 weeks and separated according to sex at 5 weeks.

Parasite

Infective larvae of *N. americanus* were originally obtained in 1983 from Dr Rajasekariah of Hindustan Ciba-Geigy Ltd, Bombay, India and were from a

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Table 1. The number of hamsters used at each time point and the dose given for each experiment

Exp. no.*	Infection dose	Days post-infection 11	35	70	105	140	175
1	20·0 ± 3·5	10	10	10	N.D.	8	N.D.
2	88·7 ± 7·3	8	10	N.D.	14	N.D.	N.D.
3	55·0 ± 6·2	6	N.D.	9	N.D.	10	N.D.
4	110·3 ± 11·2	8	10	12	6	N.D.	N.D.
5	145·1 ± 3·9	6	6	6	8	9	7
6	168·4 ± 14·7	6	11	8	5	N.D.	4
7	146·0 ± 8·0	6	7	7	N.D.	N.D.	N.D.
8	240·8 ± 5·8	6	8	8	8	N.D.	N.D.
9	100·0 ± 4·6	8	10	N.D.	12	N.D.	N.D.
10	82·3 ± 5·9	9	6	5	N.D.	N.D.	N.D.
11	226·6 ± 14·6	7	5	N.D.	6	N.D.	N.D.
12*a*	N.D.†	7	N.D.	7	N.D.	N.D.	N.D.
12*b*	94·0 ± 3·8	7	N.D.	8	N.D.	N.D.	N.D.
12*c*	N.D.†	8	N.D.	6	N.D.	N.D.	N.D.
Total animals		102	83	86	59	27	11

* Experiment 13 comprised one litter of 7 hamsters all of which were killed 35 days after infection.
† The number of larvae was determined accurately for group 12*b*. Hamsters in group 12*a* were given half this dose and hamsters in group 12*c* were given twice the dose.
N.D., Not done.

strain which had been maintained through 69 generations by infection of neonatal hamsters. This strain has since been passaged through a further 31 generations in our laboratory. Infective larvae were raised in standard Harada-Mori culture tubes at 28 °C and used when no more than 1 week old. The method used for infection of neonates has been described previously (Behnke, Wells & Brown, 1986*b*).

Autopsy

Animals were killed by an overdose of chloroform and each hamster was weighed immediately. The entire small intestine was then removed, opened longitudinally in Hanks's saline and the adult worms were picked out individually with the aid of a dissecting microscope. L_4 stages (day 11) were recovered using a modified Baermann technique, as described by Behnke, Paul & Rajasekariah (1986*a*).

Measurement of host variables

Hamsters were weighed at regular intervals and blood samples were obtained from the retro-orbital sinus, using 50 μl of heparinized capillary tubes, under trilene anaesthesia. The tubes were centrifuged for 5 min in a Hawksley Haematocrit centrifuge and the packed cell volume (PCV) was measured.

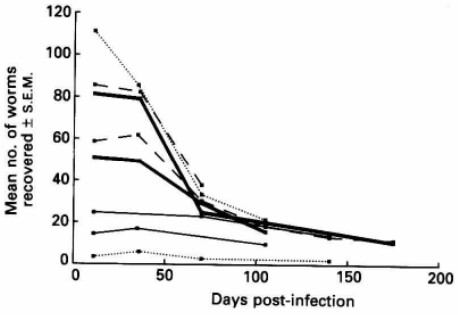

Fig. 1. Dose-dependent loss of *Necator americanus* from hamsters exposed to infection at 2 days of age. The figure summarizes the results from Exps 1–8 inclusive (each represented by a separate line). Between 4 and 14 hamsters were killed to determine the mean worm burden at each time point. Details in Table 1.

Statistical analysis of results

Worm burdens, host weight and PCVs are presented as mean values ± standard error of mean (S.E.M.) unless otherwise stated. Groups were compared by the non-parametric Mann-Whitney *U* test. Where appropriate the Spearman rank-order correlation coefficients (*rs*), the parametric correlation coefficients (*r*) and regression lines were calculated by convensional techniques to establish relationships between parameters (Sokal & Rohlf, 1969).

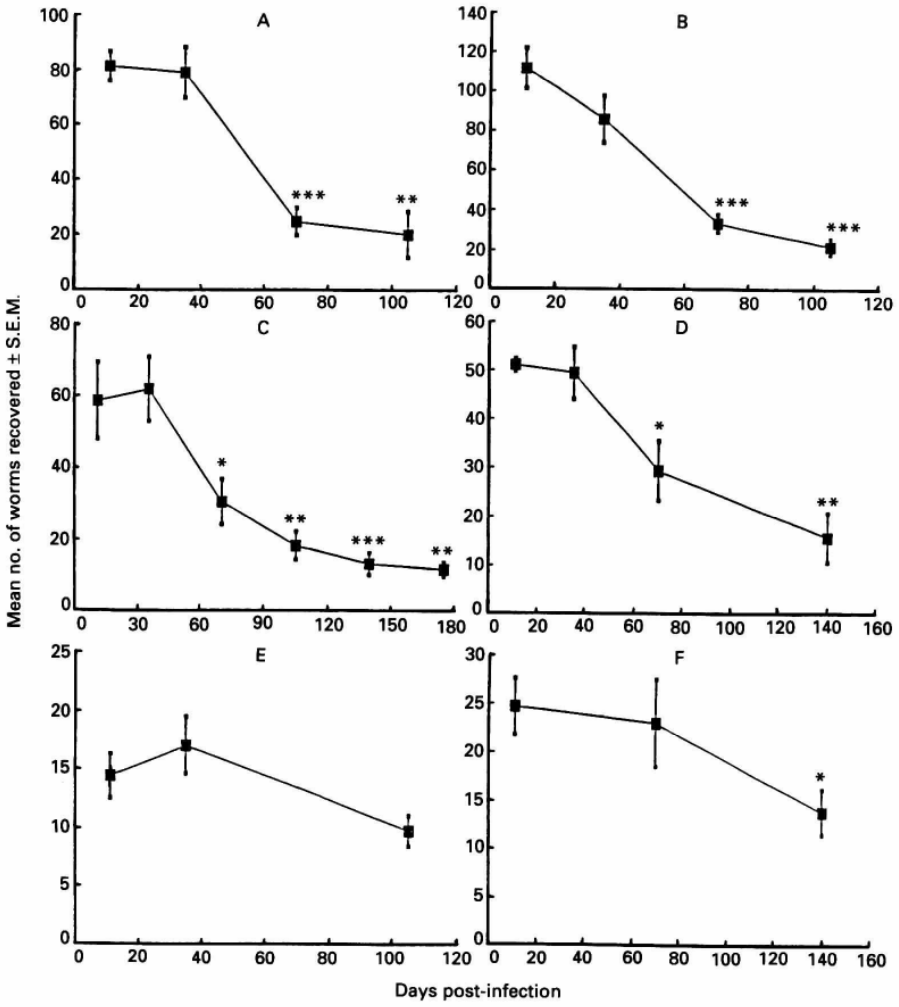

Fig. 2. Time-course of infection in six representative experiments in which the initial establishment was categorized as low (fewer than 30 worms; Fig. 2E, Exp. 2 and Fig. 2F, Exp. 3) moderate (31–60 worms; Fig.2C, Exp. 5 and Fig. 2D, Exp. 4) and high (more than 60 worms, Fig. 2A, Exp. 7 and Fig. 2B, Exp. 8) intensity. Statistical analysis of results; values for each time point were compared to that for day 11 and the levels of significance correspond to *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$; ***, $P < 0{\cdot}001$.

RESULTS

Changes in worm burden following exposure to varying doses of larvae

In a series of 12 experiments hamsters infected with doses varying from 20 to 240 larvae (Table 1) were killed at regular intervals throughout infection and established worms were counted. A summary of the results from Experiments 1–8 is given in Fig. 1 and detailed examples of representative experiments covering low (fewer than 30 worms initially established on day 11 post-infection (p.i.)), moderate (31–60 worms) and high (more than 60 worms) infection intensities are illustrated in Fig. 2. Each experiment (Exps 1–12) comprised a group of animals killed on day 11, the earliest at which a reliable assessment of worms establishing in the intestine could be made (Behnke *et al.* 1986*a*). This point was then used as a reference to enable relative loss of parasites to be calculated over the period 11–70 days p.i. (no hamsters were killed on day 70 in Exps 2, 9, 11 and 13) and the analysis of these data is presented in Fig. 3.

It is evident from Figs 1 and 2 that worm burdens were stable for a period of 5 weeks following infection, irrespective of the dose of larvae administered to hamsters. Detectable loss of parasites (but not statistically significant) in the period preceding week 5 was recorded in only one experiment (Fig. 2B) in which an exceptionally heavy worm burden developed. Loss of adult worms later in the infection period was clearly density dependent. At low infection intensities hardly any loss of worms took

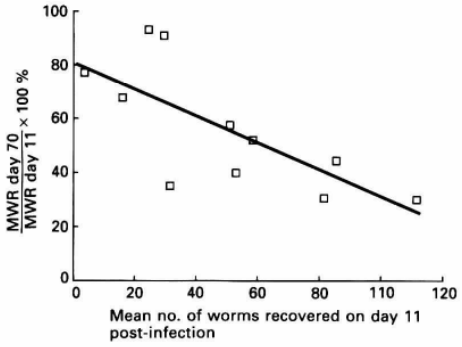

Fig. 3. Density-dependent loss of *Necator americanus* during primary infection in DSN hamsters: the percentage of initially established worms surviving until day 70 post-infection. Each point represents the MWR on day 70 expressed as a percentage of the day 11 MWR and has been plotted against the MWR on day 11 in each case. Statistical analysis of results: $y = 81{\cdot}51 - 0{\cdot}51x$; $r = -0{\cdot}72$; $P < 0{\cdot}05$. Data from Experiments 1, 3–8 and 10, and 12*a–c* inclusive.

place throughout the 10 weeks following infection (Fig. 2E and F). At higher infection intensities, such as those illustrated in Fig. 2A–D, worms were lost by week 10, stabilizing in most cases at a residual worm burden of, on average, 20 worms by week 15. The dose-dependent nature of the loss phase is emphasized by the analysis presented in Fig. 3 which shows a strong negative correlation ($r = -0{\cdot}72$, $P < 0{\cdot}05$) between percentage of worms surviving to day 70 and the initial establishment of worms on day 11.

Comparison of worm loss in male and female hamsters

Experiments 1–12 inclusive were analysed to determine whether there was any difference in the worm burdens developing in or persisting in hamsters of either sex, and 3 representative data sets are presented in Table 2. These show that, excepting day 11 worm burdens, in the experiment utilizing a very low intensity of infection, there was no statistically detectable difference in the worm burden of male versus female hamsters at any time point post-infection, irrespective of the infection intensity.

Comparison of the sex ratio of worms at various infection intensities

Data from Experiments 1–13 inclusive were pooled and analysed for any effect on the ratio of male to female worms (expressed as a percentage of male worms in the total population at a particular time point p.i.), over the course of the experimental period and in relation to the intensity of infection and sex of the host (Table 3). The percentage of male worms varied initially from 45 % to 50 % among the

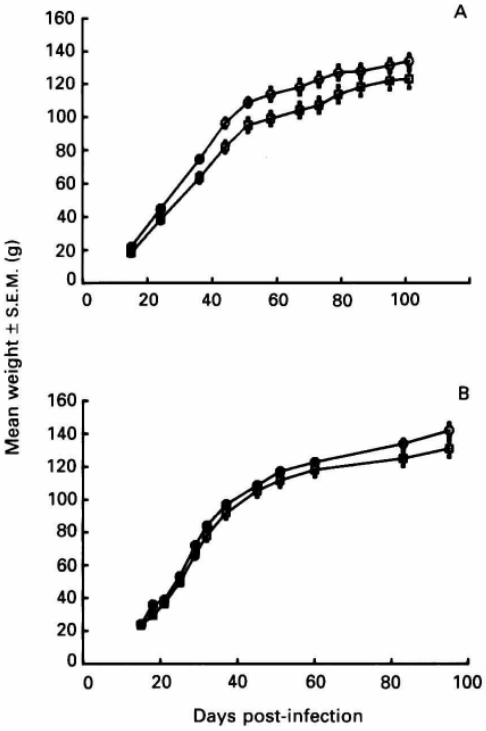

Fig. 4. Growth of hamsters following exposure to a high intensity (A, Exp. 8, $n = 8$, the MWR on day 11 was $111{\cdot}3 \pm 10{\cdot}3$) and a low intensity (B, Exp. 2, $n = 18$, the MWR on day 11 was $14{\cdot}4 \pm 1{\cdot}9$) intensity infection. Values for infected (○) and control (□) groups were compared on each day using the Mann-Whitney U-test and no significant differences were found.

combination presented in Table 3 but, as the infections progressed, there was a significant increase in the percentage of male worms comprising the total parasite population. During the period 10–20 weeks post-infection, when the majority of adult worms would have been expelled, particularly in those animals carrying the heavier worm burdens, changes in the percentage of male worms were marginal but the most impressive changes occurred by week 25, by which time male worms generally comprised over 60 % of the worm burden.

The effect of N. americanus *on the growth of hamsters*

The growth of infected and control hamsters was compared throughout infection. The groups of animals destined for the final autopsy in each experiment were selected and the litter size was adjusted to a maximum of 6 neonates immediately after infection. A corresponding group of age-matched, uninfected hamsters was used as the control in each case and both groups were weighed regularly, at least twice weekly, throughout the

Table 2. Comparison of worm recoveries from male and female hamsters at three different infection intensities

(Statistical analysis of results: groups compared using the Mann–Whitney U-test; * $P < 0.05$, all other comparisons of male versus female hamsters were not significantly different.)

Infection intensity† (Exp. no.)	Sex of hamster	Mean ± S.E.M. no. of worms recovered (n) Day 11	Day 35	Day 70
Low (1)	Male	*1·2 ± 1·0 (5)	6·8 ± 1·6 (5)	1·6 ± 1·4 (5)
	Female	*5·8 ± 1·2 (5)	5·0 ± 2·6 (5)	3·8 ± 1·9 (5)
Moderate (4)	Male	50·5 ± 4·6 (4)	50·0 ± 6·2 (5)	27·0 ± 7·0 (5)
	Female	52·0 ± 1·9 (4)	48·6 ± 4·6 (5)	31·6 ± 3·6 (7)
High (6)	Male	75·0 ± 8·0 (2)	76·0 ± 15·1 (4)	25·0 ± 7·5 (4)
	Female	84·5 ± 6·8 (4)	80·9 ± 12·5 (7)	24·8 ± 7·6 (4)

† Infection intensity was categorized as low (fewer than 30 worms), moderate (31–60 worms) and high (more than 60 worms) at day 11 after infection.

Table 3. Comparison of the percentage of male worms over time in the complete data set, in male and female hamsters, and at three different initial infection intensities

Sex of host	Intensity of infection*	Male worms as a percentage of the total worm burden ± S.E.M. Weeks post-infection 5	10	15	20	25
Both	All	47·6 ± 1·4 (90)	52·2 ± 2·3 (63)	54·3 ± 3·2 (53)	54·0 ± 5·0 (27)	66·1 ± 5·2 (11)
Male	All	48·2 ± 2·0 (43)	51·7 ± 3·8 (28)	54·2 ± 3·3 (29)	45·1 ± 7·8 (12)	63·3 ± 4·4 (5)
Female	All	47·0 ± 1·9 (47)	52·7 ± 2·8 (35)	54·4 ± 5·7 (24)	59·9 ± 5·9 (15)	68·4 ± 8·7 (6)
Both	Low	45·2 ± 2·6 (42)	39·6 ± 4·3[1] (22)	49·0 ± 5·8[2] (23)	51·5 ± 6·6 (17)	N.D.
Both	Moderate	50·1 ± 2·0 (26)	43·6 ± 3·5[1] (26)	31·8 ± 9·3[2] (5)	N.D.	48·7 ± 14·1 (4)
Both	High	49·3 ± 1·2 (22)	55·2 ± 3·6[1] (14)	58·5 ± 4·7[2] (19)	55·3 ± 7·0 (10)	66·5 ± 8·2 (7)

Statistical analysis: groups at each time point were compared using the Mann–Whitney U-test, and those with the same superscript have the following levels of significance: (1) $P < 0.06$; (2) $P < 0.05$. The relationship between the percentage of males and time after infection was analysed using the Spearman rank correlation: both sexes, all intensities of infection, $r_s = 0.213$, $P < 0.001$; male hamsters, all intensities of infection, $r_s = 0.157$, $P > 0.05$; female hamsters, all intensities of infection, $r_s = 0.268$, $P < 0.05$; both sexes, low infection intensity, $r_s = 0.083$, $P > 0.05$; both sexes, moderate infection intensity, $r_s = -0.213$, $P > 0.05$; both sexes, high infection intensities, $r_s = 0.246$, $P < 0.05$.

* The data from Experiments 1–13 were pooled and intensity of infection categorized as low (fewer than 30 worms), moderate (31–60 worms), or high (more than 60 worms) at day 11 after infection.

N.D., Note done.

experiment. Fig. 4 illustrates the results obtained in 2 experiments; one in which the infected animals were given a heavy inoculum (Fig. 4A) and the other in which a smaller dose of larvae was applied (Fig. 4B). No significant difference between the weights of infected and control animals was evident at any of the time points examined.

Data from Experiments 1–13 inclusive were then pooled and the weight of hamsters was related to the worm burden at autospy. Separate analyses were undertaken for day 11, day 35 and day 70 post-infection and the results are shown in Fig. 5. There was a significant negative correlation between worm burden and weight on days 11 ($P < 0.05$) and 35 ($P < 0.01$), but not on day 70 ($P > 0.05$). The gradient of the regression line relating weight to worm burden was small in each case (-0.033 and -0.122), indicating that despite the significant relationship, the effect of worms on the growth of infected hamsters was small.

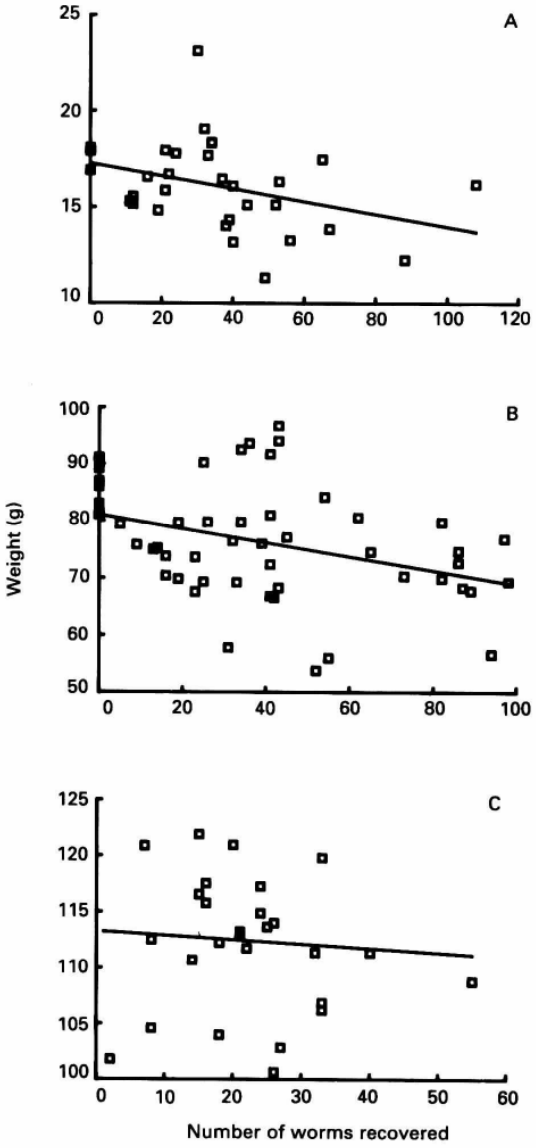

Fig. 5. Relationship between weight of hamsters and worm burdens on day 11 (A, $y = 17{\cdot}28 - 0{\cdot}033x$, $r = -0{\cdot}35$, $P = < 0{\cdot}05$), day 35 (B, $y = 81{\cdot}1 - 0{\cdot}122x$, $r = -0{\cdot}37$, $P = < 0{\cdot}01$), and day 70 (C, $y = 113{\cdot}3 - 0{\cdot}039x$, $r = -0{\cdot}057$, $P = > 0{\cdot}05$). Each symbol denotes 1 animal. Data taken from Exps 1–13.

The effect of N. americanus *on the packed cell volume of infected hamsters*

The results of two experiments in which changes in the packed cell volume (PCV) of infected and control hamsters were followed throughout the first 15 weeks are shown in Fig. 6. Again data for high and low infection intensities have been selected (Fig. 6A and B, respectively). At low infection intensity PCVs were significantly reduced relative to controls on days 39, 45, 51 and 70 post-infection, but not at other time points. A similar, but more severe, pattern was found in the more heavily infected animals, with significant anaemia detected until 106 days after infection.

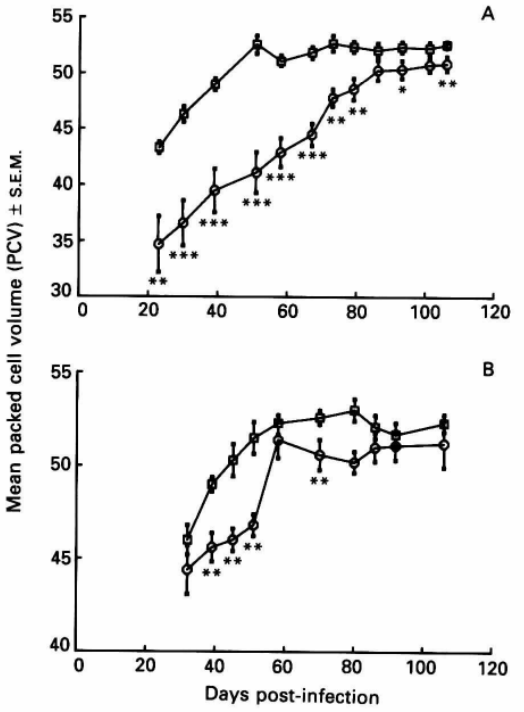

Fig. 6. Changes in the packed cell volume (PCV) during infection with *Necator americanus* in hamsters exposed to high intensity (A, Exp. 8, $n = 8$, MWR on day 11 = $111{\cdot}3 \pm 10{\cdot}3$) and low intensity (B, Exp. 3, $n = 12$, MWR on day 11 = $24{\cdot}7 \pm 2{\cdot}9$) infection. Statistical analysis of results; values for infected (○) and control (□) groups were compared on each day examined by the Mann-Whitney U-test and the levels of significance correspond to *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$; ***, $P < 0{\cdot}001$.

The relationship between PCV and worm burden was further examined by an analysis of the pooled data from all experiments in which animals were killed on days 11, 35 and 70 post-infection. At each time point there was a strong significant negative correlation ($P < 0{\cdot}001$) between PCV and worm burden. The most severe anaemia was evident on day 35 and the least severe on day 11, with gradients of $-0{\cdot}332$ and $-0{\cdot}093$ respectively, on the regression analysis.

DISCUSSION

Several potentially useful aspects of the *N. americanus*/hamster model are emphasized by the results presented in this paper. A range of initial infection levels was readily established in hamsters and the resultant pathology was clearly dependent on the intensity as well as on the duration of infection.

Three distinct phases of infection were identified.

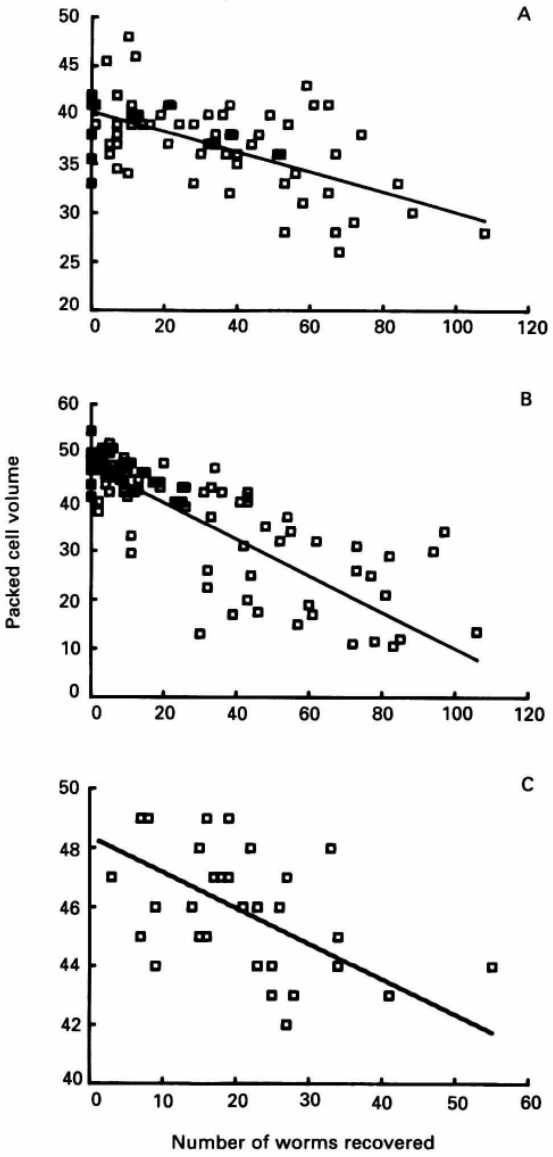

Fig. 7. Relationship between the packed cell volume (PCV) and worm burdens on day 11 (A, $y = 40{\cdot}4 - 0{\cdot}093x$, $r = -0{\cdot}559$, $P = < 0{\cdot}001$), day 35 (B, $y = 47{\cdot}6 - 0{\cdot}332$, $r = -0{\cdot}8$, $P = < 0{\cdot}001$) and day 70 (C, $y = 48{\cdot}16 - 0{\cdot}115x$, $r = -0{\cdot}608$, $P = < 0{\cdot}001$). Each symbol denotes 1 animal. Data taken from Exps 1–13.

The first, lasting 5 weeks, was characterized by stable worm burdens at all the infection intensities studied. *N. americanus* moults to the pre-adult stage in the fourth week of infection (Behnke *et al.* 1986*a*) and begins to cause the more extensive bleeding associated with the feeding activities of adult parasites. The 5 week period of relatively stable worm burdens, therefore permitted selected aspects of pathology to be assessed and related accurately to worm burdens.

The second phase of infection began 5–10 weeks after infection, when a proportion of the adult worms, directly related to the intensity of the initially established parasites, was lost (Fig. 3). Density-dependent regulation of parasitic organisms is well documented in the literature (Anderson & May, 1985; Keymer, 1982) and evidence for proportionally lesser loss of adult nematodes in hosts carrying light infections is available in *T. spiralis* (Murrell, 1985), *Nematodirus battus* (Lee & Martin, 1976 and *Trichuris muris* (Michael & Bundy, 1989).

In hasters infected with *N. americanus*, loss did not take place suddenly as for example in *T. spiralis* where, once initiated, worm expulsion is completed within a few days (Wakelin & Donachie, 1983). Rather, worms were lost over a protracted length of time, eventually stabilizing 15–20 weeks after infection. The kinetics of worm loss were similar to the sequence of events described for *Nippostrongylus brasiliensis* in neonatal rats (Jarrett, Jarrett & Urquhart, 1968*a*) in so far as loss was density dependent and worms persisted well into the adult life of the host, suggesting the possible involvement of immunological processes in regulating *N. americanus* in hamsters. Furthermore hamsters infected in neonatal life are capable of antibody responses to *N. americanus*, these coinciding with the period of worm loss. Antibody responses to the surface cuticular antigens of adult worms reached their highest titres 10–15 weeks after infection (Pritchard *et al.* 1986; Behnke & Pritchard, 1987) and vigorous antibody responses to the excretory/secretory antigens of *N. americanus* were also evident at this time (Carr & Pritchard, 1986). Interestingly the loss of *N. americanus* in hamsters coincided with the onset of patency at 6 weeks p.i. (Behnke & Pritchard, 1987), and since hookworms are known to induce exacerbated damage to the intestinal mucosa during mating activity (Beaver, Yoshida & Ash, 1964), it is conceivable that antigens released at this time induced a host-protective immune response in the host.

As far as is known, other components of intestinal immunity have not been investigated in this system and, therefore, loss from competitive interactions resulting from more parasites than the intestinal environment could sustain, cannot be excluded. Intraspecific competition between intestinal parasites is well recorded (Holmes, 1973; Keymer, 1982), particularly in tapeworms which appear to be extremely sensitive to overcrowding (Read, 1951; Roberts, 1961; Moss, 1971). However, nematodes are more resilient and, in the case of hookworms, extremely high worm burdens have been reported in other animal hosts, often with invasion of abnormal sections of the small intestine (*A. ceylanicum* in dogs; Carroll & Grove, (1984); Rep (1966)) but usually without associated worm loss (Carroll & Grove, 1984). However, the laboratory strain of *N. ameri-*

canus may not be fully adapted to the abnormal hamster host (Behnke, 1990) and a substantial proportion of the worms in heavier infections may have had a shorter life-span than the 15 years longevity reported in humans (Palmer, 1955), because of the compounding effects from overcrowding and incomplete compatibility between host and parasite. Equally it is likely that mating activities require additional nutritional resources and conceivable that these were limited in heavily infected animals resulting in the loss of the less successful worms.

The third phase of infection, was again a period of relatively stable but low intensity worm burdens. Irrespective of the initially established parasite burden, a maximum of 30 worms survived beyond week 15, and these are known to live for up to 600 days (Behnke, 1990). Nevertheless, slow loss of worms occurred throughout phase three, coincidentally with the reported reduction in fecundity during this period of infection (Behnke & Pritchard, 1987). There was also a significant increase in the proportion of male worms comprising the parasite burden during phase three. Initial values for the percentage of male worms (in week 5) were in accord with published data by Stiles & Altman (1913) and Rep (1975) (46% and 50·4% males respectively in human infections). However, studies with *Ancylostoma* sp. have generally reported a bias in favour of female worms (Roche & Patrzek, 1966) and a gradual increase in the proportion of females (*Ancylostoma caninum*) with time post-infection. Our results contrast with this latter study, since the percentage of female *N. americanus* decreased with time p.i., as was clearly evident in week 25, but bear similarity to findings with *N. brasiliensis* where the percentage of male worms in the residual worm burden is also high (Porter, 1935; Haley, 1962).

The effects of parasitic infection on host weight have been documented many times in the literature, and *N. americanus* has often been associated with marked weight loss and retarded growth in children (Keymer & Bundy, 1989), although a comparable effect in adulthood is less obvious (Holland, 1987). The results presented here, however, contrast vividly with studies on *A. ceylanicum* in hamsters (Garside & Behnke, 1989) in so far as no retardation of growth was evident in hamsters carrying either high or low intensity infections with *N. americanus*, when specific groups were followed precisely by regular weighing. When data from several experiments were pooled (Fig. 5) a significant negative correlation between weight and infection intensity was established for days 11 and 35, but not day 70 p.i. The strongest relationship was evident on day 35, indicating that the parasite's effect on host weight was more severe at a time when adult worms would have been feeding for almost 2 weeks and worm burdens were still stable.

The profound blood loss associated with *N. americanus* infection in humans has been well documented (reviewed by Roche & Layrisse (1966) and Miller (1979)). Significant blood loss also occurred in hamsters, particularly in the early stages of infection and was detectable in hamsters carrying low as well as high intensity infections. A highly significant negative correlation was established between the worm burden and PCV of hamsters on days 11 and 35 post-infection and was particularly marked, as expected on day 35. Subsequently the PCVs of infected animals returned to normal as the infection progressed towards the third, chronic phase and worm burdens declined.

There have been many reported examples of gender associated differences in the intensity of parasitic infections in man (reviewed by Goble & Konopka (1973), Bundy (1988) and Alexander & Stimson (1988)) and several field studies have found higher hookworm burdens in men than in women (Nawalinski, Schad & Chowdhury, 1978*a*, *b*; Knight & Merrett, 1981; Upatham *et al.* 1989). Differences in exposure rates between the sexes arising out of different behaviour patterns, rather than gender-associated differences in susceptibility to infection, may account for the field observations. This interpretation is supported by our observation that male and female hamsters were equally susceptible to infection and there was no difference in the rate at which worms were lost from the more heavily infected animals. However, female hamsters produce considerably fewer hookworm eggs/gram of faeces (Sen, 1972; Behnke & Pritchard, 1987) indicating that host gender does influence the fecundity of adult worms. The background to these observations was discussed by Behnke & Pritchard (1987) and the subject will form the basis of a further publication.

Finally, our experiments have established that density-dependent regulation of worm populations occurs relatively early during the course of infection with *N. americanus* in hamsters, by comparison with other models of hookworm infection but a proportion of the parasites, representing a residual worm burden survives for a considerbly longer period of time. The whole pattern of events, including the three distinct phases, bears a close resemblance to *N. brasiliensis* in neonatal rats and the loss phases associated with immune regulation of the latter species (Jarrett, Jarrett & Urquhart, 1968*b*). It is thus conceivable that immune processes were involved in our system. However, the persistence of the residual parasite burden, the marked density-dependent anaemia (as reflected in PCV) and the subtle effect on host weight, all reflect well-documented aspects of chronic human necatoriasis.

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